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Published in:
Microbial ecology and water engineering & biofilms specialist groups (MEWE2016)

Publication date:
2016

Document Version
Publisher's PDF, also known as Version of record

MICROBIAL ECOLOGY AND WATER ENGINEERING
& BIOFILMS SPECIALIST GROUPS

MEWE2016

Conference September 4th-7th, 2016, Copenhagen, Denmark

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Low-sludge age EBPR process for resource recovery – microbial and biochemical process characterization

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Keywords: low-SRT EBPR systems; phosphorus removal; sulphate reduction; microbial ecology

Introduction

New trends in water treatment focus on recycling water, energy and nutrients (N and P; Verstraete et al., 2009). Many of the recently proposed strategies involve the use of activated sludge systems operated at comparably low solid retention time, SRT (Ge et al., 2015). By operating the activated sludge process at relative low SRT wastewater resources can be separated and recovered, rather than destroyed. The main objectives of the present study are i) to describe the reactor start-up operation conditions for a low-SRT enhanced biological phosphorus removal (EBPR) system operated as a sequencing batch reactor (SBR); ii) to describe the microbial community dynamics in the SBR and identifying the key bacterial groups, affecting the process performance and the main factors controlling their microbial abundance.

Material and Methods

The EBPR system was implemented as an SBR with 8 L volume, and operated at hydraulic retention time (HRT=18 h) and under three different SRT regimes, i.e. 8, 3.5 and 3 days. The initial operation sequence was 2h of anaerobic phase, 3h of aerobic phase and 1h of settling and idle phase. The reactor was fed during the first 2 minutes of the anaerobic phase with pre-clarified wastewater supplemented with propionate and ortho-phosphate. Process performance was assessed by monitoring bulk liquid concentrations of ammonia, nitrite, nitrate, phosphate and COD, total suspended solids (TSS), pH, dissolved oxygen (DO) and the sludge volume index (SVI). Microbial diversity was analysed via 16S rRNA Illumina sequencing, quantitative fluorescence *in situ* hybridization (qFISH) and quantitative polymerase chain reaction (qPCR).

Results and Conclusions

The SBR was operated for 190 days. During the operation at SRT 8 days (first 50 days) the system efficiently removed phosphorus, COD and ammonia, whilst SVI was lower than 90 ml/g (i.e. normal operation conditions for conventional EBPR). On day 50 the system SRT was decreased to 3.5 days. Coinciding with the depletion of nitrite and nitrate at the end of the aerobic phase (i.e. effective wash out of nitrifiers) the settleability started to deteriorate (quantified as SVI). Eventually, the system was suffering extreme filamentous bulking (SVI=1100 mg/L). At that point, sulphate was analysed through a complete cycle, and results suggested that up to 30% of the influent sulphate was reduced during the anaerobic phase, which, was re-oxidized through the aerobic phase. It was hypothesized that, once nitrifiers were washed from the system and nitrate was not recycled to the beginning of the next cycle, sulphate reducing bacteria (SRBs) were able to proliferate in the system. SRBs can produce sulphur reduced compounds that are available for the filamentous bacteria *Thiothrix*, thereby leading to sludge bulking. Furthermore, it was hypothesized that the SRBs could compete with PAOs for the propionate in the anaerobic phase, thereby compromising the phosphorus uptake during the aerobic phase. Alternatively, SRBs could produce sulphide, which could inhibit phosphorus release during the anaerobic phase, thereby hindering PAOs activity. Based on these set of hypothesis, the anaerobic SRT was decreased from 1.2 to 0.88 days, intended to wash out SRBs from the reactor. These changes in the reactor operational led to a progressively decreasing SVI and increasing phosphorus removal capacity, thus removing up to 85-99% of the influent phosphate in the SBR. The system was finally run at SRT=3 days (anaerobic SRT of 0.68 days), thereby leading to ammonia accumulation in the effluent, which can be further recovered (e.g. ammonia stripping), and effective phosphorus removal via sludge wastage. The sludge can be further processed for phosphorus recovery. Further details on the process performance can be found in Valverde-Pérez (2015), whilst the biogas potential of the produced biomass in reported in Wágner et al. (2016). In Fig. 1 the distribution of the most abundant taxa at the order level are shown. It is clear that from day 50, when the system SRT was reduced, the community composition was characterized by an increase in abundance of the *Thiothrichales* order, mostly comprising of *Thiothrix spp*. This increase continued during the bulking event. From day 101 to 109 the anaerobic SRT was reduced to 0.88 days. These conditions resulted in a reduction of *Thiothrix* abundance. However, no obvious correlation in known SRB taxa (not shown) – presumed to be responsible for *Thiothrix* growth by reducing sulphate to sulphide – were found. *Rhodocyclales* were relatively abundant during good phosphorus removal and were only displaced during filamentous bulking. *Accumulibacter* , tracked via qFISH, correlated with phosphate removal suggesting that it was the main bacteria driving phosphorus removal, contrary to other low SRT EBPR systems, where *Comamonadaceae* was reported as the main PAO (Ge et al., 2015). After SVI correction and phosphorus removal restoration, the microbial composition became similar to the diversity by the end of the regime with SRT=8 days based on the H index (Fig. 1). Interestingly, results derived from Illumina sequencing, qPCR and qFISH do not agree, which highlights the importance of combining different techniques when analyzing microbial diversity of activated sludge systems.

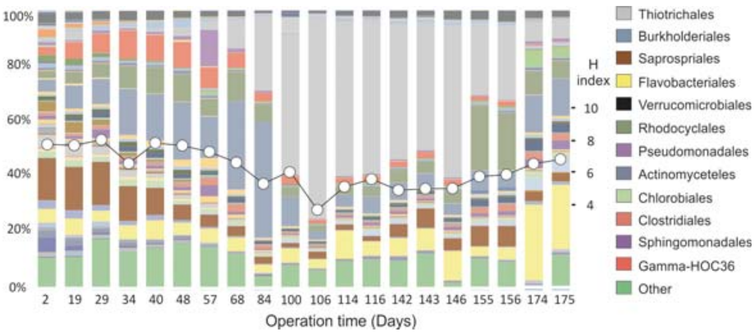

Figure 1: Order-level taxonomic classification of 16S rRNA amplicons at selected days of the reactor operation. Taxa abundance is expressed in percentage (left axis). Alpha-diversity measured as H index (right axis).

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